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ARTICLE

One third of Danish hypertrophic cardiomyopathy patients have mutations in MYH7 rod region

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Familial hypertrophic cardiomyopathy (FHC) is, in most cases, a disease of the sarcomere, caused by a mutation in one of 10 known sarcomere disease genes. More than 266 mutations have been identified since 1989. The FHC disease gene first characterized *MYH7*, encodes the cardiac β-myosin heavy chain, and contains more than 115 of these mutations. However, in most studies, only the region encoding the globular head and the hinge region of the mature cardiac β-myosin heavy chain have been investigated. Furthermore, most studies carries out screening for mutations in the most prevalent disease genes, and discontinues screening when an apparent disease-associated mutation has been identified. The aim of the present study was to screen for mutations in the rod region of the *MYH7* gene in all probands of the cohort, regardless of the known genetic status of the proband. Three disease-causing mutations were identified in the rod region in four probands using capillary electrophoresis single-strand conformation polymorphism as a screening method. All mutations were novel: N1327K, R1712W, and E1753K. Two of the probands had already been shown to carry other FHC-associated mutations. In conclusion, we show that in the Danish cohort we find one third of all *MYH7* mutations in the rod-encoding region and we find that two of the patients carrying these mutations also carry mutations in other FHC disease genes stressing the need for a complete screening of all known disease genes in FHC-patients.
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Introduction

Familial hypertrophic cardiomyopathy (FHC; MIM#192600) is considered a monogenic disorder but several disease genes have been identified, most of which are coding for sarcomere proteins. The disease may affect up to 1 in 500[1] and may be associated with syncopes, dyspnea, arrhythmia, and sudden death. FHC is a dominant trait; however, reduced penetrance and variable expressivity of FHC is often observed. The onset varies from early childhood to midlife in the same family. Several studies have shown that screening for mutations in the coding regions of at least eight disease genes results in detection of a mutation in approximately 50% of the cases. The remaining cases are most likely patients with more complex heredity or with mutations in regions not screened. *MYH7*, coding for β-myosin heavy chain, has been shown to be one of the most prevalent FHC disease genes, now with more than 115 identified mutations.[2] Two recent studies showed that a region of the *MYH7* gene, which initially was expected not to contain FHC mutations, indeed did harbour a number of disease-associated

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mutations.[3,4] These studies underscore the necessity to include all regions of the disease genes in the genetic analysis, as the current state of knowledge is based on very few published studies. In the Danish cohort of 92 families we have previously identified eight mutations in exons 3–23 of *MYH7*.[5–8] By including the rod-encoding region (exons 24–40) we identified three additional disease-associated mutations and three that may be associated with disease. As previously reported in a study of *MYBPC3* mutations,[9] several of the mutation carriers also carried mutations in other FHC-related genes.

Methods

Subjects

In total, 92 Danish patients with FHC and their families ($N = 453$) were enrolled consecutively in a study at the Copenhagen University Hospital according to previously described criteria.[10] The probands were all Caucasians; 62/38% male/female; mean age 50 years (range 5–77 years); mean maximal left ventricular wall diameter was 20.7 mm (range 15–34 in adults).[11]

Blood samples were drawn from probands and family members. Blood samples from 100 healthy blood donors were used as controls. Genomic DNA was extracted from whole blood (QIAamp Whole blood mini kit; Qiagen GmbH, Hilden, Germany).

PCR

PCR reactions were performed in a final volume of 25 μl containing 100 ng of genomic DNA, 0.25 U of AmpliTaq (Applied Biosystems, Foster City), Hotstar Taq DNA polymerase (Qiagen) or Tempase (Amplicon, Copenhagen, Denmark), and 5 pmol of gene-specific primer pairs. The primers were designed from the consensus sequence of the published sequences of the *MYH7* gene (Genbank accession numbers X52889 and AJ238393).

Primers labelled with either FAM (forward) or HEX (reverse) fluorophores were used in the PCR reactions for single-strand conformational polymorphism (SSCP) analysis by capillary electrophoresis and unlabelled primers were used for DNA-sequencing.

Mutation analysis

PCR products were analyzed using capillary array single-strand conformation polymorphism analysis (CAE-SSCP) at 18, 25, and 35°C in a 5% native GeneScan matrix (Applied Biosystems) using an ABI3100 genetic analyser (Applied Biosystems) as previously described.[12]

Fragments having aberrant CAE-SSCP profiles were sequenced by 'Big-Dye' version 2.0 cycle sequencing (Applied Biosystems), according to the manufacturer's instruction using an ABI3100 DNA sequencer. The sequences were compared with the published sequences of the *MYH7* gene (Genbank accession numbers X52889 and AJ238393) and numbered according to X52889. Probands with *MYH7* mutations were also screened for mutations in other known FHC genes (*MYBPC3*, cardiac Myosin Binding Protein C; *TPM1*, cardiac α-tropomyosin; *TNNT2*, cardiac troponin T; *TNNI3*, cardiac troponin I; *ACTC*, cardiac α-actin; *MYL2*, regulatory myosin light chain and *MYL3* essential myosin light chain) using methods described elsewhere.[10]

Database submission

All identified mutations were submitted to the Locus Specific Database for Familial Hypertrophic Cardiomyopathy (www.angis.org.au/Databases/Heart/heartbreak.html)[2].

Results and discussion

Screening for FHC mutations in the *MYH7* gene has previously been limited to the first coding exons (exons 3–23); however, recent work by Blair *et al*[3] and Richard *et al*[4] showed that this approach is not sufficient. In order to improve the genetic diagnosis for the 92 Danish FHC patients who have been screened for mutations in seven other known disease-associated FHC genes, we screened the remaining exons of *MYH7*. Since we, and others, previously found several cases of possible digenic inheritance,[9,10,13] we carried out the screening for all our probands, including those with other probable disease-associated mutations. We identified three novel mutations in the rod region in four probands (4/92; 4.3%). In two of the cases we identified a mutation in a person, previously genotyped with a mutation in the regulatory myosin light chain (*MYL2*).

Mutation screening of the rod-region identified 11 exon variants, consisting of five missense mutations, one nonsense mutation, five silent substitutions and 10 intron variants (Table 1).

The major clinical findings in the families with disease-associated variants are presented in Table 2.

Proband T (Figure1 and Table 2) was heterozygous for a C-to-A transversion in exon 30 (g_19073_C>A), resulting in an N1327K amino-acid substitution. The clinically affected individuals in the family are all heterozygous for the N1327K (Figure 1). None of the remaining probands or in 200 control chromosomes tested had the N1327K mutation. Screening of this proband in the *MYL2* gene had previously identified an A13T mutation.[10] Others have shown this mutation to be disease-associated.[14] In the present family of proband T, one of the affected individuals (T:II:4) did not have the *MYL2* A13T mutation. This person was at first considered to be a phenocopy due to severe overweight and hypertension. He did in fact carry the N1327K mutation in *MYH7*. In contrast, one of the family members (T:II:3) carrying a *MYL2* mutation, fulfilling the diagnostic criteria, did not

Table 1 Exon variants identified screening *MYH7* exons 24–40 including the flanking intron regions

Exon/intron	Nucleotide position[a]	Amino acid/nucleotide change	Proband allele frequency (N = 184)	Proband[b]	Control allele frequency (N = 200[c])
Disease-associated variants					
30	g_19073	N1327K (AAC > AAA)	0.005	T	0.000
35	g_21815	R1712W (CGG > TGG)	0.011	B, ZG	0.000
36	g_22058	E1753K (GAG > AAG)	0.005	GEA	0.000
Variants of unknown status					
30	g_19092	Q1334X (CAG > TAG)	0.005	GEA	0.000
32	g_20222	R1475C (CGC > TGC)	0.005	M	0.000
32	g_20271	S1491C[d] (TCC > TGC)	0.022	U, YH, XO[e]	0.020
32	g_20454	IVS32_−26 C > T	0.027	ZQ, XG, XQ, YF, ZV	0.000
Probable normal variants					
24	g_14438	None[d] (ATT > ATC)	0.337	—	0.270
25	g_15807	None (GCG > GCA)	0.005	XP	0.000
27	g_17849	None (GAG > GAA)	0.011	XI, GED	0.000
33	g_20525	None[d] (ACT > ACC)	0.005	XJ	0.005
35	g_21787	None (GCG > GCA)	0.092		0.130
26	g_17824	IVS26_−5 insC	0.060		0.075
28	g_18610	IVS28_+21 C > T	0.005	R	0.000
28	g_18616	IVS28_+27 T > A	0.348		0.315
29	g_18911	IVS29_+15 C > T	0.038		0.015
33	g_20627	IVS33_+24 C > T	0.016	R, X, ZB	0.005
38	g_23485	IVS38_+32 G > A	0.196		0.130
39	[f]	IVS39_−64 A > G	0.353		0.340
40	g_24264	3′-UTR_+20G > A	0.005	A	0.005
40	g_24357	3′-UTR_+113 G > A	0.065		0.070

[a]Genbank Acc. No. X52889.
[b]Probands only listed if five or less probands carried the mutation.
[c]*N* = 200 alleles from healthy blood donors.
[d]Previously published by Blair *et al* (2002).[3]
[e]Homozygous.
[f]This DNA segment was not included in the reference Genbank sequence (Acc. No. X52889), however, it is present in Genbank Acc. No. AJ238393 (Pos. 25407).

carry the N1327K mutation. The son of the proband, T:III:1, who carried both mutations, did not fulfil the diagnostic criteria, probably due to his young age. The present results suggest that either the *MYL2* mutation or the *MYH7* mutation alone may cause HCM as seen in T:II:3 and T:II:4, respectively, but the presence of both *MYL2* and the *MYH7* mutation may result in a more severe disease than either mutation alone as seen in the case of the proband.

Proband GEA (Figure1 and Table 2) carried a nonsense mutation in exon 30 changing the glutamine in codon 1334 to an amber stop codon (Q1334X) and a G-to-A transition in nucleotide 22,058 in exon 36 resulting in an amino-acid substitution (E1753K). The healthy mother (GEA:I:1) carried the Q1334X and the mildly affected father (GEA:I:2) the E1753K mutation. The affected sister also carried the E1753K mutation, but not the Q1334X (Figure 1). The E1753 residue is conserved among vertebrates. Neither the mutation causing Q1334X nor the E1753K causing mutation was found among the remaining probands or in 200 control chromosomes or in previous studies. With respect to the mutations Q1334X and E1753K, identified in one proband, only the latter mutation alone seems to be associated with the disease. However, the presence of both mutations may result in a more severe phenotype as seen in the case of the proband (GEA:II:1) compared to GEA:II:2 (Table 2).

Two probands, B and ZG, had a C-to-T transversion in exon 35, position 21815 changing an arginine residue into a tryptophan residue (R1712W). Proband B had a large family where the mutation segregates with the disease (Figure 1, Table 2), but ZG had no available relatives. R1712 is conserved in the myosin rod across mammalian species and myosin isoforms. The mutation was not detected among the remaining probands or in 200 normal control chromosomes. Proband ZG:II:1 also carried both an *MYH7* rod mutation (R1712W) and a previously detected *MYL2* mutation (N47K).[10] Also here, the *MYH7* mutation seems to be a disease-associated mutation as this particular mutation is found in all clinically affected members of another family (B). The disease-association of the *MYL2* mutation is uncertain, as no relatives of proband ZG:II:1 were available for analysis.

Table 2 Main clinical findings in families with disease-associated mutations

Family	*ID (Pedigree No.)*	*Age[a] (years)*	*MaxLVD[b] (mm)*	*R/E score[c]*	*Sokolow[d]*	*Event[e]*	*Mutation[f]*
B	**II:1**	39	26	5	Y	PTSMA	Y
	III:1	14	10	3	Y	A	Y
	II:3	47	13	0	Y	A	Y
	III:2	26	13	0	N	A	N
	III:3	24	10	0	Y	A	N
	II:5	33	12	0	Y	A	Y
	II:6	45	13	6	Y	A	Y
	II:7	32	11	0	N	A	N
	III:4	13	7	0	N	A	N
	II:9	35	11	0	Y	A	Y
	II:10	49	11	0	N	A	N
	II:11	42	9	0	Y	A	Y
	II:12	42	12	0	N	A	N
GEA	I:1	43	11	0	N	A	Y
	I:2	46	16	0	N	A	Y
	II:1	18	22	5	Y	Cardiac arrest, ICD	Y
	II:2	22	15	3	Y	A	Y
T	I:1	85	13	0	N	A	N
	II:1	46	20	5	Y	PTSMA	Y
	III:1	10	5	3	Y	A	Y
	II:3	49	23	NA	NA	S	Y
	II:4	49	14	0	N	A	Y
ZG	**II:1**	60	39	6	Y	PTSMA	Y

[a]Age denotes age of onset (proband (highlighted)) and/or age at time of clinical examination.
[b]MaxLVD denotes the maximal left ventricular wall diameter.
[c]R/E denotes Romhilt–Estes ECG score.
[d]Sokolow denotes fulfilment of Sokolow ECG criteria for hypertrophy.
[e]Event denotes the presence of cardiac events associated with FHC. PTSMA denotes percutan trans-septal myocardial ablation.
[f]Mutation denotes the presence of any mutation (Table 1). ICD = implantable cardioverter defibrillator; A = asymptomatic; S = mildly symptomatic; NA = not available; Y = Yes; N = No.

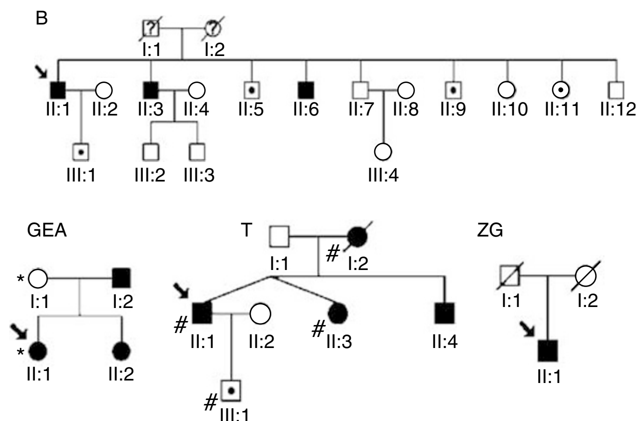

Figure 1 Pedigrees of four FHC families with mutations in the rod encoding region. The families are indicated by the appropriate letter. Filled in symbols indicate clinically affected members. Black dots indicate mutation carriers; arrows indicate index patient. (+) indicates persons fulfilling the minor diagnostic criteria; (*) indicates Q1335X nonsense carrier; and (#) indicates *MYL2* mutation carrier.

Proband M (Table 1) had a C-to-T transition in position 20,222 changing arginine-1475 into a cysteine residue (R1475C). The proband's brother also fulfilling the diagnostic criteria did not carry this variant. The brother has not been screened for other mutations. However, the R1475 residue is conserved among mammalian species and myosin isoforms, and the variant was not detected among the remaining probands or in 200 control chromosomes and had not previously been reported. The significance of this mutation is not known. It is possible that it is a disease-associated mutation; however, it cannot be ruled out that it is a rare polymorphism.

Finally, we identified 15 variants of unknown consequence as indicated in Table 1. Among these, a C-to-G transversion (g_20271_C>G) in exon 32 resulting in an S1491C change was identified in a heterozygous state in two probands (U, YH) and in a homozygous state in one, a variant identified as a relatively rare polymorphism (≈2%) in the general population in our study, and by Blair *et al*[3] (S1491C). The presence of a homozygous state in one patient is a rare event (with an estimated frequency of 0.04%), and the mutation may be a risk factor/disease modifier.

The intron variation IVS32-26_C>T seems to be over-represented among the probands (5/184 alleles, 2.7%) compared to the control group (0/200 alleles), $P = 0.049$, Fisher's exact test. This variation may be a genetic risk factor, however, more data are needed in order to verify the significance of this observation.

The head and hinge encoding region of *MYH7* contained eight disease-associated mutations and the rod region three mutations out of a total of 37% genotyped patients in the cohort. The overall frequency of *MYH7* mutations in the present study is thus considerably lower (14%) than in a recent study,[4] which finds approximately 25% in the *MYH7* gene out of a total of 63%. In the present study the rod region accounted for one-third of all disease-associated mutations identified in *MYH7* as compared to the French study, which identified 16% of the *MYH7* mutations in the rod region.[4] These apparent differences may be due to the nature of the present cohort, which is a consecutive cohort recruited in a single centre regardless of the presence of additional affected family members.

In conclusion, our data combined with the results from Blair *et al*[3] and Richard *et al*[4] show that all regions of the known FHC genes should be included in mutation screening. The discovery of individuals carrying rare mutations in more than one FHC-related gene exposes a potential risk of misinterpretation of the disease-causing mutation and may impede the genetic counselling and clinical care in some families. Alternatively, more than one disease gene may segregate in a family, resulting in the same clinical phenotype. At present it is not possible to exclude this possibility, and more genotype–phenotype studies of FHC families are therefore warranted.

Thus, the results of genotyping all probands irrespective of previously identified mutations confirmed that for a complete genetic evaluation it is necessary to perform a full genotype screening in all FHC genes for each case.

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